Amendments to the Specification:

Please delete the paragraph beginning at page 1, paragraph [001] and replace with the following rewritten paragraph:

--[001] This application is a continuation-in-part of USSN 10/672,280, filed September 26, 2003 which claims benefit under 35 U.S.C. ~~§199(e)~~ 119(e) to USSNs ~~10/672,280, filed September 26, 2003, 10/379,392, filed March 3, 2003,~~ 60/477,839 filed June 12, 2003; 60/467,606, filed May 2, 2003; 60/414,433 filed September 27, 2002; and 60/442,301 filed January 23, 2003, and is a continuation-in-part of USSN 10/379,392, filed March 3, 2003, all of which are expressly incorporated by reference in their entirety.--

VW 7/24/07

Please delete the paragraph beginning at page ~~19~~ 18, paragraph [039] and replace with the following rewritten paragraph:

--[039] Figure 3. The amino acid sequence of the heavy chain of the antibody alemtuzumab (Campath®, a registered trademark of Ilex Pharmaceuticals LP) (SEQ ID NO:1), illustrating positions numbered sequentially (2 lines above the amino acid sequence) and positions numbered according to the EU index as in Kabat (2 lines below the amino acid sequence. The approximate beginnings of Ig domains VH1, Cγ1, the hinge, Cγ2, and Cγ3 are also labeled above the sequential numbering. Polymorphisms have been observed at a number of Fc positions, including but not limited to Kabat 270, 272, 312, 315, 356, and 358, and thus slight differences between the presented sequence and sequences in the prior art may exist.--

Please delete the paragraph beginning at page 19, paragraph [041] and replace with the following rewritten paragraph:

--[041] Figure 5. The human IgG1 Fc sequence (SEQ ID NO:2) showing positions relevant to the design of the Fc variant experimental library. The sequence includes the hinge region, domain Cγ2, and domain Cγ3. Residue numbers are according to the EU index as in Kabat. Positions relevant to the experimental library are underlined. Because of observed polymorphic mutations at a number of Fc positions, slight differences between the presented sequence and sequences in the literature may exist.--

VW 7/24/07

Please delete the paragraph beginning at page ~~25~~ 24, paragraph [074] and replace with the following rewritten paragraph:

-- [074] Figures 38a – 38c. Sequences showing improved anti-CD20 antibodies. The light and heavy chain sequences of rituximab (SEQ ID NOs:3 and 4) are presented in Figure 38a and Figure 38b respectively, and are taken from translated Sequence 3 of US 5,736,137. Relevant positions in Figure

38b are bolded, including S239, V240, V264I, E272, K274, N297, S298, K326, A330, and I332. Figure 38c shows the improved anti-CD20 antibody heavy chain sequences, with variable positions designated in bold as X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, Z_1, and Z_2 (SEQ ID NO:5). The table below the sequence provides possible substitutions for these positions. The improved anti-CD20 antibody sequences comprise at least one non-WT amino acid selected from the group of possible substitutions for X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8. These improved anti-CD20 antibody sequences may also comprise a substitution Z_1 and/or Z_2. These positions are numbered according to the EU index as in Kabat, and thus do not correspond to the sequential order in the sequence. --

VW 7/24/07

Please delete the paragraph beginning at page ~~45~~ 44, paragraph [0118] and replace with the following rewritten paragraph:

--[118] A variety of linkers may find use in the present invention to generate Fc fusions (see definition above) or antibody- or Fc fusion- conjugates (see definition below). By "linker", "linker sequence", "spacer", "tethering sequence" or grammatical equivalents thereof, herein is meant a molecule or group of molecules (such as a monomer or polymer) that connects two molecules and often serves to place the two molecules in a preferred configuration. A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. Choosing a suitable linker for a specific case where two polypeptide chains are to be connected depends on various parameters, including but not limited to the nature of the two polypeptide chains (e.g., whether they naturally oligomerize), the distance between the N- and the C-termini to be connected if known, and/or the stability of the linker towards proteolysis and oxidation. Furthermore, the linker may contain amino acid residues that provide flexibility. Thus, the linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. Suitable lengths for this purpose include at least one and not more than 30 amino acid residues. Preferably, the linker is from about 1 to 30 amino acids in length, with linkers of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 19 and 20 amino acids in length being preferred. In addition, the amino acid residues selected for inclusion in the linker peptide should exhibit properties that do not interfere significantly with the activity of the polypeptide. Thus, the linker peptide on the whole should not exhibit a charge that would be inconsistent with the activity of the polypeptide, or interfere with internal folding, or form bonds or other interactions with amino acid

The linker may be cleavable, facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari et al., 1992, Cancer Research 52: 127-131) may be used. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use to link the Fc variants of the present invention to a fusion partner to generate an Fc fusion, or to link the antibodies and Fc fusions of the present invention to a conjugate.--

VW 7/24/07

Please delete the paragraph beginning at page 73, paragraph [0501] and replace with the following rewritten paragraph: 176

--[176] Fc variants may be operably linked to a fusion partner to enable targeting of the expressed protein, purification, screening, display, and the like. Fusion partners may be linked to the Fc variant sequence via a linker sequences. The linker sequence will generally comprise a small number of amino acids, typically less than ten, although longer linkers may also be used. Typically, linker sequences are selected to be flexible and resistant to degradation. As will be appreciated by those skilled in the art, any of a wide variety of sequences may be used as linkers. For example, a common linker sequence comprises the amino acid sequence GGGGS (SEQ ID NO:7). A fusion partner may be a targeting or signal sequence that directs Fc variant protein and any associated fusion partners to a desired cellular location or to the extracellular media. As is known in the art, certain signaling sequences may target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. A fusion partner may also be a sequence that encodes a peptide or protein that enables purification and/or screening. Such fusion partners include but are not limited to polyhistidine tags (His-tags) (for example H_6 and H_{10} or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (e.g. Ni^{+2} affinity columns)), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like). As will be appreciated by those skilled in the art, such tags may be useful for purification, for screening, or both. For example, an Fc variant may be purified using a His-tag by immobilizing it to a Ni^{+2} affinity column, and then after purification the same His-tag may be used to immobilize the antibody to a Ni^{+2} coated plate to perform an ELISA or other binding assay (as described below). A fusion partner may enable the use of a selection method to screen Fc variants (see below). Fusion partners that enable a variety of selection methods are well-known in the art, and all of these find use in the present invention. For example, by fusing the members of an Fc variant library to the gene III protein, phage display can be employed (Kay *et al.*,

Phage display of peptides and proteins: a laboratory manual, Academic Press, San Diego, CA, 1996; Lowman *et al.*, 1991, *Biochemistry* 30:10832-10838; Smith, 1985, *Science* 228:1315-1317). Fusion partners may enable Fc variants to be labeled. Alternatively, a fusion partner may bind to a specific sequence on the expression vector, enabling the fusion partner and associated Fc variant to be linked covalently or noncovalently with the nucleic acid that encodes them. For example, USSN 09/642,574; USSN 10/080,376; USSN 09/792,630; USSN 10/023,208; USSN 09/792,626; USSN 10/082,671; USSN 09/953,351; USSN 10/097,100; USSN 60/366,658; PCT WO 00/22906; PCT WO 01/49058; PCT WO 02/04852; PCT WO 02/04853; PCT WO 02/08023; PCT WO 01/28702; and PCT WO 02/07466 describe such a fusion partner and technique that may find use in the present invention.--

VW 7/24/07 **Please delete page ~~89~~ 90 since it is a blank page.**

VW 7/24/07 **Please delete the paragraph beginning at page ~~120~~ 122, paragraph [204] and replace with the following rewritten paragraph:**

--**[204]** The results of the design calculations presented above in Tables 1 – 60 were used to construct a series of Fc variant libraries for experimental production and screening. Experimental libraries were designed in successive rounds of computational and experimental screening. Design of subsequent Fc libraries benefitted from feedback from prior libraries, and thus typically comprised combinations of Fc variants that showed favorable properties in the previous screen. The entire set of Fc variants that were constructed and experimentally tested is shown in Table 61. In this table, row 1 lists the variable positions, and the rows that follow indicate the amino acids at those variable positions for WT and the Fc variants. For example, variant 18 (SEQ ID NO:26) has the following four mutations: F241E, F243Y, V262T, and V264R. The variable position residues that compose this set of Fc variants are illustrated structurally in Figure 4, and are presented in the context of the human IgG1 Fc sequence (SEQ ID NO:2) in Figure 5.--

VW 7/24/07 **Please delete Table 61 beginning at page ~~121~~ 123 and replace with the following rewritten table:**

-- **Table 61**

Variant	SEQ ID NO	Substitution(s)	Variant	SEQ ID NO	Substitution(s)
1	SEQ ID NO:9	V264A	50	SEQ ID NO:55	Y296Q
2	SEQ ID NO:10	V264L	51	SEQ ID NO:56	S298T
3	SEQ ID NO:11	V264I	52	SEQ ID NO:57	S298N
4	SEQ ID NO:12	F241W	53	SEQ ID NO:58	T299I
5	SEQ ID NO:13	F241L	54	SEQ ID NO:59	A327S
6	SEQ ID NO:14	F243W	55	SEQ ID NO:60	A327N
7	SEQ ID NO:15	F243L	56	SEQ ID NO:61	S267Q/A327S

8	SEQ ID NO:16	F241L/F243L/V262I/V264I	57	SEQ ID NO:62	S267L/A327S
9	SEQ ID NO:17	F241W/F243W	58	SEQ ID NO:63	A327L
10	SEQ ID NO:18	F241W/F243W/V262A/V264A	59	SEQ ID NO:64	P329F
11	SEQ ID NO:19	F241L/V262I	60	SEQ ID NO:65	A330L
12	SEQ ID NO:20	F243L/V264I	61	SEQ ID NO:66	A330Y
13	SEQ ID NO:21	F243L/V262I/V264W	62	SEQ ID NO:67	I332D
14	SEQ ID NO:22	F241Y/F243Y/V262T/V264T	63	SEQ ID NO:68	N297S
15	SEQ ID NO:23	F241E/F243R/V262E/V264R	64	SEQ ID NO:69	N297D
16	SEQ ID NO:24	F241E/F243Q/V262T/V264E	65	SEQ ID NO:70	N297S/I332E
17	SEQ ID NO:25	F241R/F243Q/V262T/V264R	66	SEQ ID NO:71	N297D/I332E
18	SEQ ID NO:26	F241E/F243Y/V262T/V264R	67	SEQ ID NO:72	N297E/I332E
19	SEQ ID NO:27	L328M	68	SEQ ID NO:73	D265Y/N297D/I332E
20	SEQ ID NO:28	L328E	69	SEQ ID NO:74	D265Y/N297D/T299L/I332E
21	SEQ ID NO:29	L328F	70	SEQ ID NO:75	D265F/N297E/I332E
22	SEQ ID NO:30	I332E	71	SEQ ID NO:76	L328I/I332E
23	SEQ ID NO:31	L328M/I332E	72	SEQ ID NO:77	L328Q/I332E
24	SEQ ID NO:32	P244H	73	SEQ ID NO:78	I332N
25	SEQ ID NO:33	P245A	74	SEQ ID NO:79	I332Q
26	SEQ ID NO:34	P247V	75	SEQ ID NO:80	V264T
27	SEQ ID NO:35	W313F	76	SEQ ID NO:81	V264F
28	SEQ ID NO:36	P244H/P245A/P247V	77	SEQ ID NO:82	V240I
29	SEQ ID NO:37	P247G	78	SEQ ID NO:83	V263I
30	SEQ ID NO:38	V264I/I332E	79	SEQ ID NO:84	V266I
31	SEQ ID NO:39	F241E/F243R/V262E/V264R/I332E	80	SEQ ID NO:85	T299A
32	SEQ ID NO:40	F241E/F243Q/V262T/V264E/I332E	81	SEQ ID NO:86	T299S
33	SEQ ID NO:41	F241R/F243Q/V262T/V264R/I332E	82	SEQ ID NO:87	T299V
34	SEQ ID NO:42	F241E/F243Y/V262T/V264R/I332E	83	SEQ ID NO:88	N325Q
35	SEQ ID NO:43	S298A	84	SEQ ID NO:89	N325L
36	SEQ ID NO:44	S298A/I332E	85	SEQ ID NO:90	N325I
37	SEQ ID NO:45	S298A/E333A/K334A	86	SEQ ID NO:91	S239D
41	SEQ ID NO:46	S239E/I332E	87	SEQ ID NO:92	S239N
42	SEQ ID NO:47	S239Q/I332E	88	SEQ ID NO:93	S239F
43	SEQ ID NO:48	S239E	89	SEQ ID NO:94	S239D/I332D
44	SEQ ID NO:49	D265G	90	SEQ ID NO:95	S239D/I332E
45	SEQ ID NO:50	D265N	91	SEQ ID NO:96	S239D/I332N
46	SEQ ID NO:51	S239E/D265G	92	SEQ ID NO:97	S239D/I332Q
47	SEQ ID NO:52	S239E/D265N	93	SEQ ID NO:98	S239E/I332D
48	SEQ ID NO:53	S239E/D265Q	94	SEQ ID NO:99	S239E/I332N
49	SEQ ID NO:54	Y296E	95	SEQ ID NO:100	S239E/I332Q

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VW 7/24/07

Please delete Table 61 (continued) beginning at page ~~122~~ 124 and replace with the following rewritten table:

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Table 61 (continued)

Variant	SEQ ID NO	Substitution(s)	Variant	SEQ ID NO	Substitution(s)

VW 7/24/07

Please delete Table 61 (continued) beginning at page ~~123~~ 125 and replace with the following rewritten table:

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Table 61 (continued)

Variant	SEQ ID NO	Substitution(s)	Variant	SEQ ID NO	Substitution(s)
186	SEQ ID NO:191	N297D/I332E/S239D/D265I	231	SEQ ID NO:236	E269N
187	SEQ ID NO:192	N297D/I332E/S239D/D265L	232	SEQ ID NO:237	D270Q
188	SEQ ID NO:193	N297D/I332E/S239D/D265F	233	SEQ ID NO:238	D270T
189	SEQ ID NO:194	N297D/I332E/S239D/D265Y	234	SEQ ID NO:239	D270H
190	SEQ ID NO:195	N297D/I332E/S239D/D265H	235	SEQ ID NO:240	E272S
191	SEQ ID NO:196	N297D/I332E/S239D/D265T	236	SEQ ID NO:241	E272K
192	SEQ ID NO:197	N297D/I332E/V264E	237	SEQ ID NO:242	E272I
193	SEQ ID NO:198	N297D/I332E/Y296D	238	SEQ ID NO:243	E272Y
194	SEQ ID NO:199	N297D/I332E/Y296E	239	SEQ ID NO:244	V273I
195	SEQ ID NO:200	N297D/I332E/Y296N	240	SEQ ID NO:245	K274T
196	SEQ ID NO:201	N297D/I332E/Y296Q	241	SEQ ID NO:246	K274E
197	SEQ ID NO:202	N297D/I332E/Y296H	242	SEQ ID NO:247	K274R
198	SEQ ID NO:203	N297D/I332E/Y296T	243	SEQ ID NO:248	K274L
199	SEQ ID NO:204	N297D/I332E/T299V	244	SEQ ID NO:249	K274Y
200	SEQ ID NO:205	N297D/I332E/T299I	245	SEQ ID NO:250	F275W
201	SEQ ID NO:206	N297D/I332E/T299L	246	SEQ ID NO:251	N276S
202	SEQ ID NO:207	N297D/I332E/T299F	247	SEQ ID NO:252	N276E
203	SEQ ID NO:208	N297D/I332E/T299H	248	SEQ ID NO:253	N276R
204	SEQ ID NO:209	N297D/I332E/T299E	249	SEQ ID NO:254	N276L
205	SEQ ID NO:210	N297D/I332E/A330Y	250	SEQ ID NO:255	N276Y
206	SEQ ID NO:211	N297D/I332E/S298A/A330Y	251	SEQ ID NO:256	Y278T
207	SEQ ID NO:212	S239D/I332E/A330Y	252	SEQ ID NO:257	Y278E
208	SEQ ID NO:213	S239N/I332E/A330Y	253	SEQ ID NO:258	Y278K
209	SEQ ID NO:214	S239D/I332E/A330L	254	SEQ ID NO:259	Y278W
210	SEQ ID NO:215	S239N/I332E/A330L	255	SEQ ID NO:260	E283R
211	SEQ ID NO:216	I332E/V264I/S298A	256	SEQ ID NO:261	V302I
212	SEQ ID NO:217	I332E/S239D/S298A	257	SEQ ID NO:262	E318R
213	SEQ ID NO:218	I332E/S239N/S298A	258	SEQ ID NO:263	K320T
214	SEQ ID NO:219	S239D/I332E/V264I	259	SEQ ID NO:264	K320D
215	SEQ ID NO:220	S239D/I332E/V264I/S298A	260	SEQ ID NO:265	K320I
216	SEQ ID NO:221	S239D/I332E/V264I/A330L	261	SEQ ID NO:266	K322T
217	SEQ ID NO:222	L328N	262	SEQ ID NO:267	K322H
218	SEQ ID NO:223	L328H	263	SEQ ID NO:268	V323I
219	SEQ ID NO:224	S239D/I332E/A330I	264	SEQ ID NO:269	S324T
220	SEQ ID NO:225	N297D/I332E/S239D/A330L	265	SEQ ID NO:270	S324D
221	SEQ ID NO:226	P230A	266	SEQ ID NO:271	S324R
222	SEQ ID NO:227	E233D	267	SEQ ID NO:272	S324I
223	SEQ ID NO:228	P230A/E233D	268	SEQ ID NO:273	S324V
224	SEQ ID NO:229	P230A/E233D/I332E	269	SEQ ID NO:274	S324L
225	SEQ ID NO:230	S267T	270	SEQ ID NO:275	S324Y
226	SEQ ID NO:231	S267H	271	SEQ ID NO:276	K326L
227	SEQ ID NO:232	S267D	272	SEQ ID NO:277	K326I
228	SEQ ID NO:233	S267N	273	SEQ ID NO:278	K326T
229	SEQ ID NO:234	E269T	274	SEQ ID NO:279	A327D

230	SEQ ID NO:235	E269L	275	SEQ ID NO:280	A327T

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VW 7/24/07 **Please delete Table 61 (continued) beginning at page 124 and replace with the following rewritten table:**

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Table 61 (continued)

Variant	SEQ ID NO	Substitution(s)	Variant	SEQ ID NO	Substitution(s)
276	SEQ ID NO:281	A330S	290	SEQ ID NO:295	T335Y
277	SEQ ID NO:282	A330W	291	SEQ ID NO:296	L234I/L235D
278	SEQ ID NO:283	A330M	292	SEQ ID NO:297	V240I/V266I
279	SEQ ID NO:284	P331V	293	SEQ ID NO:298	S239D/A330Y/I332E/L234I
280	SEQ ID NO:285	P331H	294	SEQ ID NO:299	S239D/A330Y/I332E/L235D
281	SEQ ID NO:286	E333T	295	SEQ ID NO:300	S239D/A330Y/I332E/V240I
282	SEQ ID NO:287	E333H	296	SEQ ID NO:301	S239D/A330Y/I332E/V264T
283	SEQ ID NO:288	E333I	297	SEQ ID NO:302	S239D/A330Y/I332E/V266I
284	SEQ ID NO:289	E333Y	298	SEQ ID NO:303	S239D/A330Y/I332E/K326E
285	SEQ ID NO:290	K334I	299	SEQ ID NO:304	S239D/A330Y/I332E/K326T
286	SEQ ID NO:291	K334T	300	SEQ ID NO:305	S239D/N297D/I332E/A330Y
287	SEQ ID NO:292	K334F	301	SEQ ID NO:306	S239D/N297D/I332E/A330Y /F241S/F243H/V262T/V264T
288	SEQ ID NO:293	T335D	302	SEQ ID NO:307	S239D/N297D/I332E/L235D
289	SEQ ID NO:294	T335R	303	SEQ ID NO:308	S239D/N297D/I332E/K326E

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VW 7/24/07 **Please delete Table 62 beginning at page 127 and replace with the following rewritten table:**

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Table 62

Variant	SEQ ID NO	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
1	SEQ ID NO:9	V264A	0.53		
2	SEQ ID NO:10	V264L	0.56		
3	SEQ ID NO:11	V264I	1.43		
4	SEQ ID NO:12	F241W	0.29		
5	SEQ ID NO:13	F241L	0.26		
6	SEQ ID NO:14	F243W	0.51		
7	SEQ ID NO:15	F243L	0.51		
8	SEQ ID NO:16	F241L/F243L/V262I/V264I	0.09		
9	SEQ ID NO:17	F241W/F243W	0.07		
10	SEQ ID NO:18	F241W/F243W/V262A/V264A	0.04		
11	SEQ ID NO:19	F241L/V262I	0.06		
12	SEQ ID NO:20	F243L/V264I	1.23		

13	SEQ ID NO:21	F243L/V262I/V264W	0.02		
14	SEQ ID NO:22	F241Y/F243Y/V262T/V264T	0.05		
15	SEQ ID NO:23	F241E/F243R/V262E/V264R	0.05		
16	SEQ ID NO:24	F241E/F243Q/V262T/V264E	0.07		
17	SEQ ID NO:25	F241R/F243Q/V262T/V264R	0.02		
18	SEQ ID NO:26	F241E/F243Y/V262T/V264R	0.05		
19	SEQ ID NO:27	L328M	0.21		
20	SEQ ID NO:28	L328E	0.12		
21	SEQ ID NO:29	L328F	0.24		
22	SEQ ID NO:30	I332E	6.72	3.93	1.71
23	SEQ ID NO:31	L328M/I332E	2.60		
24	SEQ ID NO:32	P244H	0.83		
25	SEQ ID NO:33	P245A	0.25		
26	SEQ ID NO:34	P247V	0.53		
27	SEQ ID NO:35	W313F	0.88		
28	SEQ ID NO:36	P244H/P245A/P247V	0.93		
29	SEQ ID NO:37	P247G	0.54		
30	SEQ ID NO:38	V264I/I332E	12.49	1.57*	7.96
31	SEQ ID NO:39	F241E/F243R/V262E/V264R/I332E	0.19		
32	SEQ ID NO:40	F241E/F243Q/V262T/V264E/I332E			
33	SEQ ID NO:41	F241R/F243Q/V262T/V264R/I332E			
34	SEQ ID NO:42	F241E/F243Y/V262T/V264R/I332E	0.10		
35	SEQ ID NO:43	S298A	2.21		
36	SEQ ID NO:44	S298A/I332E	21.73		
37	SEQ ID NO:45	S298A/E333A/K334A	2.56		
41	SEQ ID NO:46	S239E/I332E	5.80	3.49	1.66
42	SEQ ID NO:47	S239Q/I332E	6.60	4.68	1.41
43	SEQ ID NO:48	S239E	10.16		
44	SEQ ID NO:49	D265G	<0.02		
45	SEQ ID NO:50	D265N	<0.02		
46	SEQ ID NO:51	S239E/D265G	<0.02		
47	SEQ ID NO:52	S239E/D265N	0.02		
48	SEQ ID NO:53	S239E/D265Q	0.05		
49	SEQ ID NO:54	Y296E	0.73	1.11	0.66
50	SEQ ID NO:55	Y296Q	0.52	0.43	1.21

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VW 7/24/07

Please delete Table 62 (continued) beginning at page ~~128~~ 130 and replace with the following rewritten table:

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Table 62 (continued)

Variant	SEQ ID NO	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold

97	SEQ ID NO:102	S239N/I332E	14.21		
98	SEQ ID NO:103	S239N/I332N	0.43		

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VW 7/24/07

Please delete Table 62 (continued) beginning at page ~~129~~ 131 and replace with the following rewritten table:

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Table 62 (continued)

Variant	SEQ ID NO	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
99	SEQ ID NO:104	S239N/I332Q	0.56		
100	SEQ ID NO:105	S239Q/I332D	5.05		
101	SEQ ID NO:106	S239Q/I332N	0.39		
102	SEQ ID NO:107	S239Q/I332Q	0.59		
103	SEQ ID NO:108	K326E	3.85		
104	SEQ ID NO:109	Y296D	0.62		
105	SEQ ID NO:110	Y296N	0.29		
106	SEQ ID NO:111	F241Y/F243Y/V262T/V264T/ N297D/I332E	0.15		
107	SEQ ID NO:112	A330Y/I332E	12.02	4.40	2.73
108	SEQ ID NO:113	V264I/A330Y/I332E	12.00	3.54	3.39
109	SEQ ID NO:114	A330L/I332E	10.34	2.03	5.09
110	SEQ ID NO:115	V264I/A330L/I332E	11.15	1.79	6.23
111	SEQ ID NO:116	L234D	0.21		
112	SEQ ID NO:117	L234E	1.34	2.21	0.61
113	SEQ ID NO:118	L234N	0.56	1.39	0.40
114	SEQ ID NO:119	L234Q	0.37		
115	SEQ ID NO:120	L234T	0.35		
116	SEQ ID NO:121	L234H	0.33		
117	SEQ ID NO:122	L234Y	1.42	1.08	1.31
118	SEQ ID NO:123	L234I	1.55	1.14	1.36
119	SEQ ID NO:124	L234V	0.38		
120	SEQ ID NO:125	L234F	0.30		
121	SEQ ID NO:126	L235D	1.66	3.63	0.46
122	SEQ ID NO:127	L235S	1.25		
123	SEQ ID NO:128	L235N	0.40		
124	SEQ ID NO:129	L235Q	0.51		
125	SEQ ID NO:130	L235T	0.52		
126	SEQ ID NO:131	L235H	0.41		
127	SEQ ID NO:132	L235Y	1.19	10.15	0.12
128	SEQ ID NO:133	L235I	1.10	0.94	1.17
129	SEQ ID NO:134	L235V	0.48		
130	SEQ ID NO:135	L235F	0.73	3.53	0.21
131	SEQ ID NO:136	S239T	1.34		

132	SEQ ID NO:137	S239H	0.20		
133	SEQ ID NO:138	S239Y	0.21		
134	SEQ ID NO:139	V240A	0.70	0.14	5.00
135	SEQ ID NO:140	V240T			
136	SEQ ID NO:141	V240M	2.06	1.38	1.49
137	SEQ ID NO:142	V263A			
138	SEQ ID NO:143	V263T	0.43		
139	SEQ ID NO:144	V263M	0.05		
140	SEQ ID NO:145	V264M	0.26		
141	SEQ ID NO:146	V264Y	1.02	0.27	3.78
142	SEQ ID NO:147	V266A	<0.02		
143	SEQ ID NO:148	V266T	0.45		
144	SEQ ID NO:149	V266M	0.62		
145	SEQ ID NO:150	E269H	<0.02		
146	SEQ ID NO:151	E269Y	0.12		

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VW 7/24/07 **Please delete Table 62 (continued) beginning at page 130 and replace with the following rewritten table:** 132

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Table 62 (continued)

Variant	SEQ ID NO	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
147	SEQ ID NO:152	E269F	0.16		
148	SEQ ID NO:153	E269R	0.05		
149	SEQ ID NO:154	Y296S	0.12		
150	SEQ ID NO:155	Y296T	<0.02		
151	SEQ ID NO:156	Y296L	0.22		
152	SEQ ID NO:157	Y296I	0.09		
153	SEQ ID NO:158	A298H	0.27		
154	SEQ ID NO:159	T299H	<0.02		
155	SEQ ID NO:160	A330V	0.43		
156	SEQ ID NO:161	A330I	1.71	0.02	85.5
157	SEQ ID NO:162	A330F	0.60		
158	SEQ ID NO:163	A330R	<0.02		
159	SEQ ID NO:164	A330H	0.52		
160	SEQ ID NO:165	N325D	0.41		
161	SEQ ID NO:166	N325E	<0.02		
162	SEQ ID NO:167	N325A	0.11		
163	SEQ ID NO:168	N325T	1.10		
164	SEQ ID NO:169	N325V	0.48		
165	SEQ ID NO:170	N325H	0.73		
166	SEQ ID NO:171	L328D/I332E	1.34		
167	SEQ ID NO:172	L328E/I332E	0.20		

168	SEQ ID NO:173	L328N/I332E	<0.02		
169	SEQ ID NO:174	L328Q/I332E	0.70		
170	SEQ ID NO:175	L328V/I332E	2.06		
171	SEQ ID NO:176	L328T/I332E	1.10		
172	SEQ ID NO:177	L328H/I332E	<0.02		
173	SEQ ID NO:178	L328I/I332E	3.49		
174	SEQ ID NO:179	L328A	0.20		
175	SEQ ID NO:180	I332T	0.72		
176	SEQ ID NO:181	I332H	0.46		
177	SEQ ID NO:182	I332Y	0.76		
178	SEQ ID NO:183	I332A	0.89		
179	SEQ ID NO:184	S239E/V264I/I332E	15.46		
180	SEQ ID NO:185	S239Q/V264I/I332E	2.14		
181	SEQ ID NO:186	S239E/V264I/A330Y/I332E	8.53		
182	SEQ ID NO:187	S239E/V264I/S298A/A330Y/I332E			
183	SEQ ID NO:188	S239D/N297D/I332E	0.28		
184	SEQ ID NO:189	S239E/N297D/I332E	0.06		
185	SEQ ID NO:190	S239D/D265V/N297D/I332E	0.03		
186	SEQ ID NO:191	S239D/D265I/N297D/I332E	0.01		
187	SEQ ID NO:192	S239D/D265L/N297D/I332E	<0.02		
188	SEQ ID NO:193	S239D/D265F/N297D/I332E	<0.02		
189	SEQ ID NO:194	S239D/D265Y/N297D/I332E	0.02		
190	SEQ ID NO:195	S239D/D265H/N297D/I332E	0.04		
191	SEQ ID NO:196	S239D/D265T/N297D/I332E	<0.02		
192	SEQ ID NO:197	V264E/N297D/I332E	0.05		
193	SEQ ID NO:198	Y296D/N297D/I332E			
194	SEQ ID NO:199	Y296E/N297D/I332E	<0.02		

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VW 7/24/07

Please delete Table 62 (continued) beginning at page 131 and replace with the following rewritten table:

133

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Table 62 (continued)

Variant	SEQ ID NO	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
195	SEQ ID NO:200	Y296N/N297D/I332E	0.04		
196	SEQ ID NO:201	Y296Q/N297D/I332E	<0.02		
197	SEQ ID NO:202	Y296H/N297D/I332E	<0.02		
198	SEQ ID NO:203	Y296T/N297D/I332E	<0.02		
199	SEQ ID NO:204	N297D/T299V/I332E	<0.02		
200	SEQ ID NO:205	N297D/T299I/I332E	<0.02		
201	SEQ ID NO:206	N297D/T299L/I332E	<0.02		
202	SEQ ID NO:207	N297D/T299F/I332E	<0.02		
203	SEQ ID NO:208	N297D/T299H/I332E	<0.02		
204	SEQ ID NO:209	N297D/T299E/I332E	<0.02		

VW 7/24/07 **Please delete Table 62 (continued) beginning at page ~~133~~ 135 and replace with the following rewritten table:**

-- **Table 62 (continued)**

Variant	SEQ ID NO	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
246	SEQ ID NO:251	N276S	0.41		
247	SEQ ID NO:252	N276E	0.87		
248	SEQ ID NO:253	N276R	0.66		
249	SEQ ID NO:254	N276L	1.07		
250	SEQ ID NO:255	N276Y	0.56		
251	SEQ ID NO:256	Y278T	1.87		
252	SEQ ID NO:257	Y278E	0.90		
253	SEQ ID NO:258	Y278K			
254	SEQ ID NO:259	Y278W	0.41		
255	SEQ ID NO:260	E283R	0.67		
256	SEQ ID NO:261	V302I	1.01		
257	SEQ ID NO:262	E318R	1.06		
258	SEQ ID NO:263	K320T			
259	SEQ ID NO:264	K320D			
260	SEQ ID NO:265	K320I			
261	SEQ ID NO:266	K322T			
262	SEQ ID NO:267	K322H			
263	SEQ ID NO:268	V323I	0.83		
264	SEQ ID NO:269	S324T			
265	SEQ ID NO:270	S324D	1.07		
266	SEQ ID NO:271	S324R	0.71		
267	SEQ ID NO:272	S324I	1.15		
268	SEQ ID NO:273	S324V	1.17		
269	SEQ ID NO:274	S324L	<0.02		
270	SEQ ID NO:275	S324Y	0.98		
271	SEQ ID NO:276	K326L			
272	SEQ ID NO:277	K326I	1.43		
273	SEQ ID NO:278	K326T	1.88		
274	SEQ ID NO:279	A327D	<0.02		
275	SEQ ID NO:280	A327T	<0.02		
276	SEQ ID NO:281	A330S			
277	SEQ ID NO:282	A330W			
278	SEQ ID NO:283	A330M			
279	SEQ ID NO:284	P331V			
280	SEQ ID NO:285	P331H			
281	SEQ ID NO:286	E333T	0.78		
282	SEQ ID NO:287	E333H	0.75		
283	SEQ ID NO:288	E333I			
284	SEQ ID NO:289	E333Y			

285	SEQ ID NO:290	K334I			
286	SEQ ID NO:291	K334T			
287	SEQ ID NO:292	K334F			
288	SEQ ID NO:293	T335D	2.79		
289	SEQ ID NO:294	T335R	2.58		
290	SEQ ID NO:295	T335Y	1.56		
291	SEQ ID NO:296	L234I/L235D	0.07		
292	SEQ ID NO:297	V240I/V266I	1.72		
293	SEQ ID NO:298	S239D/A330Y/I332E/L234I	22.39		
294	SEQ ID NO:299	S239D/A330Y/I332E/L235D	7.04		
295	SEQ ID NO:300	S239D/A330Y/I332E/V240I	27.97		
296	SEQ ID NO:301	S239D/A330Y/I332E/V264T	17.72		
297	SEQ ID NO:302	S239D/A330Y/I332E/V266I			
298	SEQ ID NO:303	S239D/A330Y/I332E/K326E	64.14		
299	SEQ ID NO:304	S239D/A330Y/I332E/K326T	59.03		
300	SEQ ID NO:305	S239D/N297D/I332E/A330Y	<0.02		
301	SEQ ID NO:306	S239D/N297D/I332E/A330Y/ F241S/F243H/V262T/V264T	<0.02		
302	SEQ ID NO:307	S239D/N297D/I332E/L235D			
303	SEQ ID NO:308	S239D/N297D/I332E/K326E			

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VW 7/24/07 **Please delete the paragraph beginning at page ~~134~~ 137, paragraph [211] and replace with the following rewritten paragraph:**

--[211] Example 3: Selectively enhanced binding to FcγRs

A number of promising Fc variants with optimized properties were obtained from the FcγRIIIa and FcγRIIb screen. Table 62 provides Fc variants that bind more tightly to FcγRIIIa, and thus are candidates for improving the effector function of antibodies and Fc fusions. These include a number of variants that comprise substitutions at 239, 264, 272, 274, 330, and 332. Figures 13a and 13b show AlphaScreen™ binding data for some of these Fc variants. The majority of these Fc variants provide substantially greater FcγRIIIa binding enhancements over S298A/E333A/K334A (SEQ ID NO:45).--

VW 7/24/07 **Please delete the paragraph and table beginning at page ~~136~~ 137, paragraph [~~218~~] 214 and replace with the following rewritten paragraph:**

--[214] Some of the most promising Fc variants of the present invention for enhancing effector function have both substantial increases in affinity for FcγRIIIa and favorable FcγRIIIa-fold:FcγRIIb-fold ratios. These include, for example, S239D/I332E (SEQ ID NO:95) (FcγRIIIa-fold = 56, FcγRIIIa-fold:FcγRIIb-fold = 3), S239D/A330Y/I332E (SEQ ID NO:212) (FcγRIIIa-fold = 130), S239D/A330L/I332E (SEQ ID NO:214) (FcγRIIIa-fold = 139, FcγRIIIa-fold:FcγRIIb-fold = 18), and S239D/S298A/I332E (SEQ ID

NO:217) (FcγRIIIa-fold = 295, FcγRIIIa-fold:FcγRIIb-fold = 48). Figure 17 shows AlphaScreen™ binding data for these and other Fc variants to human V158 FcγRIIIa.--

VW 7/24/07 **Please delete the paragraph and table beginning at page ~~135~~ 139, paragraph [218] and replace with the following rewritten paragraph:**

--[218] Example 5: Aglycosylated Fc variants

As discussed, one goal of the current experiments was to obtain optimized aglycosylated Fc variants. Several Fc variants provide significant progress towards this goal. Because it is the site of glycosylation, substitution at N297 results in an aglycosylated Fc. Whereas all other Fc variants that comprise a substitution at N297 completely ablate FcγR binding, N297D/I332E (SEQ ID NO:71) has significant binding affinity for FcγRIIIa, shown in Table 62 and illustrated in Figure 20. The exact reason for this result is uncertain in the absence of a high-resolution structure for this variant, although the computational screening predictions suggest that it is potentially due to a combination of new favorable Fc/FcγR interactions and favorable electrostatic properties. Indeed other electrostatic substitutions are envisioned for further optimization of aglycosylated Fc. Table 62 shows that other aglycosylated Fc variants such as S239D/N297D/I332E (SEQ ID NO:188) and N297D/A330Y/I332E (SEQ ID NO:210) provide binding enhancements that bring affinity for FcγRIIIa within 0.28- and 0.43-fold respectively of glycosylated WT alemtuzumab. Combinations of these variants with other Fc variants that enhance FcγR binding are contemplated, with the goal of obtaining aglycosylated Fc variants that bind one or more FcγRs with affinity that is approximately the same as or even better than glycosylated parent Fc. An additional set of promising Fc variants provide stability and solubility enhancements in the absence of carbohydrate. Fc variants that comprise substitutions at positions 241, 243, 262, and 264, positions that do not mediate FγR binding but do determine the interface between the carbohydrate and Fc, ablate FγR binding, presumably because they perturb the conformation of the carbohydrate. In deglycosylated form, however, Fc variants F241E/F243R/V262E/V264R (SEQ ID NO:23), F241E/F243Q/V262T/V264E (SEQ ID NO:24), F241R/F243Q/V262T/V264R (SEQ ID NO:25), and F241E/F243Y/V262T/V264R (SEQ ID NO:26) show stronger binding to FcγRIIIa than in glycosylated form, as shown by the AlphaScreen™ data in Figure 21. This result indicates that these are key positions for optimization of the structure, stability, solubility, and function of aglycosylated Fc. Together these results suggests that protein engineering can be used to restore the favorable functional and solution properties of antibodies and Fc fusions in the absence of carbohydrate, and pave the way for aglycosylated antibodies and Fc fusions with favorable solution properties and full functionality that comprise substitutions at these and other Fc positions.--

VW 7/24/07 **Please delete Table 63 beginning at page ~~137~~ 140 and replace with the following rewritten table:**

--

Table 63

	SPR V158 FcγRIIIa		SPR F158 FcγRIIIa		AlphaScreen™ V158 FcγRIIIa		AlphaScreen™ F158 FcγRIIIa	
	Kd (nM)	**Fold**	**Kd (nM)**	**Fold**	IC50 (nM)	**Fold**	IC50 (nM)	**Fold**
WT (SEQ ID NO:2)	68		730		6.4		17.2	
V264I (SEQ ID NO:11)	64	1.1	550	1.3	4.5	1.4	11.5	1.5
I332E (SEQ ID NO:30)	31	2.2	72	10.1	1.0	6.4	2.5	6.9
V264I/I332E (SEQ ID NO:38)	17	4.0	52	14.0	0.5	12.8	1.1	15.6
S298A (SEQ ID NO:43)	52	1.3	285	2.6	2.9	2.2	12.0	1.4
S298A/E333A/ K334A (SEQ ID NO:45)	39	1.7	156	4.7	2.5	2.6	7.5	2.3

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VW 7/24/07 **Please delete the paragraph beginning at page ~~137~~ 140, paragraph [221] and replace with the following rewritten paragraph:**

--[221] The SPR data corroborate the improvements to FcγRIIIa affinity observed by AlphaScreen™ assay. Table 63 further indicates the superiority of V264I/I332E (SEQ ID NO:38) and I332E (SEQ ID NO:30) over S298A (SEQ ID NO:43) and S298A/E333A/K334A (SEQ ID NO:45); whereas S298A/E333A/K334A (SEQ ID NO:45) improves Fc binding to V158 and F158 FcγRIIIa by 1.7-fold and 4.7-fold respectively, I332E (SEQ ID NO:30) shows binding enhancements of 2.2-fold and 10.1-fold respectively, and V264I/I332E (SEQ ID NO:38) shows binding enhancements of 4.0-fold and 14-fold respectively. Also worth noting is that the affinity of V264I/I332E (SEQ ID NO:38) for F158 FcγRIIIa (52 nM) is better than that of WT for the V158 allotype (68 nM), suggesting that this Fc variant, as well as those with even greater improvements in binding, may enable the clinical efficacy of antibodies for the low responsive patient population to achieve that currently possible for high responders. The correlation between the SPR and AlphaScreen™ binding measurements are shown in Figures 23a – 23d. Figures 23a and 23b show the Kd - IC50 correlations for binding to V158 FcγRIIIa and F158 FcγRIIIa respectively, and Figures 23c and 23d show the fold-improvement correlations for binding to V158 FcγRIIIa and F158 FcγRIIIa respectively. The good fits of these data to straight lines ($r^2 = 0.9$, $r^2 = 0.84$, $r^2 = 0.98$, and $r^2 =$

0.90) support the accuracy the AlphaScreen™ measurements, and validate its use for determining the relative FcγR binding affinities of Fc variants.--

VW 7/24/07 **Please delete the paragraph beginning at page ~~138~~ 141, paragraph [222] and replace with the following rewritten paragraph:**

--[222] SPR data were also acquired for binding of select trastuzumab Fc variants to human V158 FcγRIIIa, F158 FcγRIIIa, and FcγRIIb. These data are shown in Table 64. The Fc variants tested show substantial binding enhancements to the activating receptor FcγRIIIa, with over 100-fold tighter binding observed for interaction of S239D/I332E/S298A (SEQ ID NO:217) with F158 FcγRIIIa. Furthermore, for the best FcγRIIIa binders, F158 FcγRIIIa/FcγRIIb ratios of 3 – 4 are observed.--

VW 7/24/07 **Please delete Table 64 beginning at page ~~138~~ 141 and replace with the following rewritten table:**

--

Table 64

	SPR V158 FcγRIIIa		SPR F158 FcγRIIIa		SPR FcγRIIb	
	Kd (nM)	Fold	**Kd (nM)**	Fold	IC50 (nM)	**Fold**
WT (SEQ ID NO:2)	363.5		503		769	
V264I/I332E (SEQ ID NO:38)	76.9	4.7	252	2.0	756	1.0
V264I/I332E/A330L (SEQ ID NO:115)	113.0	3.2	88	5.7	353	2.2
S239D/I332E/A330L (SEQ ID NO:214)	8.2	44.3	8.9	56.5	46	16.7
S239D/I332E/S298A (SEQ ID NO:217)	8.7	41.8	4.9	102.7	32	24.0
S239D/I332E/V264I/A330L (SEQ ID NO:221)	12.7	28.6	6.3	79.8	35	22.0

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VW 7/24/07 **Please delete the paragraph beginning at page ~~138~~ 141, paragraph [~~222~~ 224] and replace with the following rewritten paragraph:**

--[224] ADCC assays were run on Fc variant and WT alemtuzumab using DoHH-2 lymphoma target cells. Figure 24a is a bar graph showing the ADCC of these proteins at 10 ng/ml antibody. Results show that alemtuzumab Fc variants I332E (SEQ ID NO:30), V264I (SEQ ID NO:11), and I332E/V264I (SEQ ID NO:38) have substantially enhanced ADCC compared to WT alemtuzumab, with the relative ADCC enhancements proportional to their binding improvements to FcγRIIIa as indicated by AlphaScreen™ assay and SPR. The dose dependence of ADCC on antibody concentration is shown in Figure 24b. The binding data were normalized to the minimum and maximum fluorescence signal for each particular curve, provided by the baselines at low and high antibody concentrations respectively. The data were fit

to a sigmoidal dose-response model using nonlinear regression, represented by the curve in the figure. The fits enable determination of the effective concentration 50% (EC50) (i.e. the concentration required for 50% effectiveness), which provides the relative enhancements to ADCC for each Fc variant. The EC50s for these binding data are analogous to the IC50s obtained from the AlphaScreen™ competition data, and derivation of these values is thus analogous to that described in Example 2 and Figure 11. In Figure 24b, the log(EC50)s, obtained from the fits to the data, for WT (SEQ ID NO:2), V264I/I332E (SEQ ID NO:38), and S239D/I332E (SEQ ID NO:95) alemtuzumab are 0.99, 0.60, and 0.49 respectively, and therefore their respective EC50s are 9.9, 4.0, and 3.0. Thus V264I/I332E (SEQ ID NO:38) and S239E/I332E (SEQ ID NO:95) provide a 2.5-fold and 3.3-fold enhancement respectively in ADCC over WT alemtuzumab using PBMCs expressing heterozygous V158/F158 FcγRIIIa. These data are summarized in Table 65 below.-- 142

VW 7/24/07 **Please delete Table 65 beginning at page ~~139~~ and replace with the following rewritten table:**

-- **Table 65**

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
WT (SEQ ID NO:2)	0.99	9.9	
V264I/I332E (SEQ ID NO:38)	0.60	4.0	2.5
S239D/I332E (SEQ ID NO:95)	0.49	3.0	3.3

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142

VW 7/24/07 **Please delete the paragraph beginning at page ~~139~~, paragraph [225] and replace with the following rewritten paragraph:**

--[225] In order to determine whether these ADCC enhancements are broadly applicable to antibodies, select Fc variants were evaluated in the context of trastuzumab and rituximab. ADCC assays were run on Fc variant and WT trastuzumab using two breast carcinoma target cell lines BT474 and Sk-Br-3. Figure 25a shows a bar graph illustrating ADCC at 1 ng/ml antibody. Results indicate that V264I (SEQ ID NO:11) and V264I/I332E (SEQ ID NO:38) trastuzumab provide substantially enhanced ADCC compared to WT trastuzumab, with the relative ADCC enhancements proportional to their binding improvements to FcγRIIIa as indicated by AlphaScreen™ assay and SPR. Figures 25b and 25c show the dose dependence of ADCC on antibody concentration for select Fc variants. The EC50s obtained from the fits of these data and the relative fold-improvements in ADCC are provided in Table 66 below. Significant ADCC improvements are observed for I332E (SEQ ID NO:30) trastuzumab when combined with A330L (SEQ ID NO:65) and A330Y(SEQ ID NO:66). Furthermore, S239D/A330L/I332E (SEQ ID NO:214) provides

a substantial ADCC enhancement, greater than 300-fold for PBMCs expressing homozygous F158/F158 FcγRIIIa, relative to WT trastuzumab and S298A/E333A/K334A(SEQ ID NO:45), consistent with the FcγR binding data observed by the AlphaScreen™ assay and SPR.--

VW 7/24/07 **Please delete Table 66 beginning at page ~~140~~ 143 and replace with the following rewritten table:**

--

Table 66

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
Figure 25b			
WT (SEQ ID NO:2)	1.1	11.5	
I332E (SEQ ID NO:30)	0.34	2.2	5.2
A330Y/I332E (SEQ ID NO:112)	-0.04	0.9	12.8
A330L/I332E (SEQ ID NO:114)	0.04	1.1	10.5
Figure 25d			
WT (SEQ ID NO:2)	-0.15	0.71	
S298A/E333A/K334A (SEQ ID NO:45)	-0.72	0.20	3.6
S239D/A330L/I332E (SEQ ID NO:214)	-2.65	0.0022	323

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VW 7/24/07 **Please delete the paragraph beginning at page ~~140~~ 143, paragraph [226] and replace with the following rewritten paragraph:**

--[226] ADCC assays were run on V264I/I332E (SEQ ID NO:38), WT (SEQ ID NO:2), and S298A/~~D~~E333A/K334A (SEQ ID NO:45) rituximab using WIL2-S lymphoma target cells. Figure 26a presents a bar graph showing the ADCC of these proteins at 1 ng/ml antibody. Results indicate that V264I/I332E (SEQ ID NO:38) rituximab provides substantially enhanced ADCC relative to WT rituximab, as well as superior ADCC to S298A/~~D~~E333A/K334A (SEQ ID NO:45), consistent with the FcγRIIIa binding improvements observed by AlphaScreen™ assay and SPR. Figures 26b and 26c show the dose dependence of ADCC on antibody concentration for select Fc variants. The EC50s obtained from the fits of these data and the relative fold-improvements in ADCC are provided in Table 67 below. As can be seen S239D/I332E/A330L (SEQ ID NO:214) rituximab provides greater than 900-fold enhancement in EC50 over WT for PBMCs expressing homozygous F158/F158 FcγRIIIa. The differences in ADCC enhancements observed for alemtuzumab, trastuzumab, and rituximab are likely due to the use of different PBMCs, different antibodies, and different target cell lines.--

143

Please delete Table 67 beginning at page 140 and replace with the following rewritten table:

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Table 67

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
Figure 26b			
WT (SEQ ID NO:2)	0.23	1.7	
S298A/E333A/K334A (SEQ ID NO:45)	-0.44	0.37	4.6
V264I/I332E (SEQ ID NO:38)	-0.83	0.15	11.3
Figure 26c			
WT (SEQ ID NO:2)	0.77	5.9	
S239D/I332E/A330L (SEQ ID NO:214)	-2.20	0.0063	937

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144

Please delete the paragraph beginning at page 141, paragraph [228] and replace with the following rewritten paragraph:

--[228] A critical parameter governing the clinical efficacy of anti-cancer antibodies is the expression level of target antigen on the surface of tumor cells. Thus a major clinical advantage of Fc variants that enhance ADCC may be that it enables the targeting of tumors that express lower levels of antigen. In To test this hypothesis, WT and Fc variant trastuzumab antibodies were tested for their ability to mediate ADCC against different cell lines expressing varying levels of the Her2/neu target antigen. ADCC assays were run with various cell lines expressing amplified to low levels of Her2/neu receptor, including Sk-Br-3 ($1x10^6$ copies), SkOV3 ($\sim 1x10^5$), OVCAR3($\sim 1x10^4$), and MCF-7 ($\sim 3x10^3$ copies), using the DELFIA EuTDA Cytotoxicity kit (PerkinElmer, Boston, MA). Target cells were loaded with BATDA in batch for 25 minutes, washed multiple times with medium and seeded at 10,000 cells per well in 96-well plates. Target cells were opsonized for 15 minutes with various antibodies and concentrations (final conc. ranging from 100 ng/ml to .0316 ng/ml in ½ log steps, including no treatment control). Human PBMCs, isolated from buffy-coat and allotyped as homozygous F158/F158 FcγRIIIa were then added to opsonized cells at 25-fold excess and co-cultured at 37°C for 4 hrs. Thereafter, plates were centrifuged, supernatants were removed and treated with Eu3+ solution, and relative fluorescence units (correlating to the level of cell lysis) were measured using a Packard Fusion™ α-FP HT reader (PerkinElmer, Boston, MA). The experiment was carried out in triplicates. Figure 28 shows the ADCC data comparing WT and Fc variant trastuzumab against the four different Her2/neu$^+$ cell lines. The S239D/I332E (SEQ ID NO:95) and S239D/I332E/A330L (SEQ ID NO:214) variants provide substantial ADCC enhancements over WT

trastuzumab at high, moderate, and low expression levels of target antigen. This result suggests that the Fc variants of the present invention may broaden the therapeutic window of anti-cancer antibodies.--

VW 7/24/07 **Please delete the paragraph beginning at page ~~142~~ 145, paragraph [230] and replace with the following rewritten paragraph:**

--[230] Example 8. ADCP of Fc Variants

Another important FcγR-mediated effector function is ADCP. Phagocytosis of target cancer cells may not only lead to the immediate destruction of target cells, but because phagocytosis is a potential mechanism for antigen uptake and processing by antigen presenting cells, enhanced ADCP may also improve the capacity of the antibody or Fc fusion to elicit an adaptive immune response. The ability of the Fc variants of the present invention to mediate ADCP was therefore investigated. Monocytes were isolated from heterozygous V158/F158 FcγRIIIa PBMCs using a Percoll gradient. After one week in culture in the presence of 0.1 ng/ml, differentiated macrophages were detached with EDTA/PBS- and labeled with the lipophilic fluorophore, PKH26, according to the manufacturer's protocol (Sigma, St Louis, Mo). Sk-Br-3 target cells were labeled with PKH67 (Sigma, St Louis, Mo), seeded in a 96-well plate at 20,000 cells per well, and treated with designated final concentrations of WT or Fc variant trastuzumab. PKH26-labeled macrophages were then added to the opsonized, labeled Sk-Br-3 cells at 20,000 cells per well and the cells were co-cultured for 18 hrs before processing cells for analysis of dual label flow cytometry. Percent phagocytosis was determined as the number of cells co-labeled with PKH76 and PKH26 (macrophage + Sk-Br-3) over the total number of Sk-Br-3 in the population (phagocytosed + non-phagocytosed) after 10,000 counts. Figure 30 shows data comparing WT and Fc variant trastuzumab at various antibody concentrations. The results indicate that the S239D/I332E/A330L (SEQ ID NO:214) variant provides a significant enhancement in ADCP over WT trastuzumab.--

VW 7/24/07 **Please delete the paragraph beginning at page ~~142~~ 145, paragraph [231] and replace with the following rewritten paragraph:**

--[231] Example 9. Complement binding and activation by Fc variants

Complement protein C1q binds to a site on Fc that is proximal to the FcγR binding site, and therefore it was prudent to determine whether the Fc variants have maintained their capacity to recruit and activate complement. The AlphaScreen™ assay was used to measure binding of select Fc variants to the complement protein C1q. The assay was carried out with biotinylated WT alemtuzumab antibody attached to streptavidin donor beads as described in Example 2, and using C1q coupled directly to acceptor beads. Binding data of V264I (SEQ ID NO:11), I332E (SEQ ID NO:30), S239E (SEQ ID

NO:48), and V264I/I332E (SEQ ID NO:38) rituximab shown in Figure 31a indicate that C1q binding is uncompromised. Cell-based CDC assays were also performed on select Fc variants to investigate whether Fc variants maintain the capacity to activate complement. Alamar Blue was used to monitor lysis of Fc variant and WT rituximab-opsonized WIL2- S lymphoma cells by human serum complement (Quidel, San Diego, CA). The data in Figure 31b show that CDC is uncompromised for the Fc variants S239E (SEQ ID NO:48), V264I (SEQ ID NO:11), and V264I/I332E (SEQ ID NO:38) rituximab. In contrast, Figure 31c shows that CDC of the Fc variant S239D/I332E/A330L (SEQ ID NO:214) is completely ablated, whereas the S239D/I332E variant (SEQ ID NO:95) mediates CDC that is comparable to WT rituximab. These results indicate that protein engineering can be used to distinguish between different effector functions. Such control will not only enable the generation of antibodies and Fc fusions with properties tailored for a desired clinical outcome, but also provide a unique set of reagents with which to experimentally investigate effector function biology.--

VW 7/24/07 **Please delete the paragraph beginning at page ~~143~~ 146, paragraph [233] and replace with the following rewritten paragraph:**

--[233] Example 11. Capacity of Fc variants to bind mouse FcγRs

Optimization of Fc to nonhuman FcγRs may be useful for experimentally testing Fc variants in animal models. For example, when tested in mice (for example nude mice, SCID mice, xenograft mice, and/or transgenic mice), antibodies and Fc fusions that comprise Fc variants that are optimized for one or more mouse FcγRs may provide valuable information with regard to clinical efficacy, mechanism of action, and the like. In order to evaluate whether the Fc variants of the present invention may be useful in such experiments, affinity of select Fc variants for mouse FcγRIII was measured using the AlphaScreen™ assay. The AlphaScreen™ assay was carried out using biotinylated WT alemtuzumab attached to streptavidin donor beads as described in Example 2, and GST-tagged mouse FcγRIII bound to glutathione chelate acceptor beads, expressed and purified as described in Example 2. These binding data are shown in Figures 34a and 34b in the context of alemtuzumab and trastuzumab respectively. Results show that some Fc variants that enhance binding to human FcγRIIIa also enhance binding to mouse FcγRIII. The enhancement of mouse effector function by the Fc variants was investigated by performing the aforementioned cell-based ADCC assays using mouse rather than human PBMC's. Figure 35 shows that the S239D/I332E/A330L (SEQ ID NO:214) trastuzumab variant provides substantial ADCC enhancement over WT in the presence of mouse immune cells. This result indicates that the Fc variants of the present invention, or other Fc variants that are optimized for nonhuman FcγRs, may find use in experiments that use animal models.--

VW 7/24/07 **Please delete the paragraph beginning at page ~~144~~ 147, paragraph [235] and replace with the following rewritten paragraph:**

--[235] Example 13. Enhancement of Fc variants in Fucose Minus Strain.

Combinations of the Fc variants of the present invention with other Fc modifications are contemplated with the goal of generating novel antibodies or Fc fusions with optimized properties. It may be beneficial to combine the Fc variants of the present invention with other Fc modifications, including modifications that alter effector function or interaction with one or more Fc ligands. Such combination may provide additive, synergistic, or novel properties in antibodies or Fc fusions. For example, a number of methods exist for engineering different glycoforms of Fc that alter effector function. Engineered glycoforms may be generated by a variety of methods known in the art, many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region. One method for engineering Fc glycoforms is to express the Fc polypeptide in a cell line that generates altered glycoforms, for example Lec-13 CHO cells. In order to investigate the properties of Fc variants combined with engineered glycoforms, WT and V209 (S239D/I332E/A330L(SEQ ID NO:214)) trastuzumab were expressed in Lec-13 CHO cells and purified as described above. Figure 37a shows AlphaScreen™ binding data comparing the binding to human V158 FcγRIIIa by WT and V209 trastuzumab expressed in 293T, CHO, and Lec-13 cells. The results show that there is substantial synergy between the engineered glycoforms produced by this cell line and the Fc variants of the present invention. The cell-based ADCC assay, shown in Figure 37b, supports this result. Together these data indicate that other Fc modifications, particularly engineered glycoforms, may be combined with the Fc variants of the present invention to generate antibodies and Fc fusions with optimized effector functions.--

VW 7/24/07 **Please delete the paragraph beginning at page ~~145~~ 147, paragraph [236] and replace with the following rewritten paragraph:**

--[236] Example 14. Therapeutic application of Fc variants

A number of Fc variants described in the present invention have significant potential for improving the therapeutic efficacy of anticancer antibodies. For illustration purposes, a number of Fc variants of the present invention have been incorporated into the sequence of the antibody rituximab. The WT rituximab light chain (SEQ ID NO:3) and heavy chain (SEQ ID NO:4), described in US 5,736,137, are provided in Figures 38a and 38b. The improved anti-CD20 antibody sequences (SEQ ID NO:5) are provided in Figure 38c. The improved anti-CD20 antibody sequences comprise at least non-WT amino acid selected from the group consisting of X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8. These improved anti-CD20 antibody sequences may also comprise a substitution Z_1 and/or Z_2. The use of rituximab here is solely an example,

and is not meant to constrain application of the Fc variants to this antibody or any other particular antibody or Fc fusion.--

VW 7/24/07 **Please delete Table 68 beginning at page ~~146~~ 149 and replace with the following rewritten table:**

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Table 68

Variant	SEQ ID NO	Substitution(s)	FcγRI	FcγRIIa	FcγRIIb	FcγRIIc	FcγRIIIa	FcRn	C1q
111	SEQ ID NO:116	L234D	0.54	1.28	2.91	2.99	2.88	1.60	1.69
112	SEQ ID NO:117	L234E	0.51	0.71	1.65	1.86	2.05	0.15	1.05
113	SEQ ID NO:118	L234N	0.11	0.07	0.90	1.11	0.20	1.64	1.02
114	SEQ ID NO:119	L234Q	0.22	1.51	2.25	2.18	0.11	3.73	0.48
115	SEQ ID NO:120	L234T	0.18	0.95	1.19	2.00	0.25	0.99	1.78
116	SEQ ID NO:121	L234H	0.07	1.75	3.24	1.32	0.09	1.01	1.04
117	SEQ ID NO:122	L234Y	0.36	0.99	1.06	1.82	0.51	0.78	1.15
118	SEQ ID NO:123	L234I	0.48	1.12	1.98	0.60	0.76	1.30	1.97
119	SEQ ID NO:124	L234V	0.86	1.81	3.23	0.93	1.83	1.33	1.39
120	SEQ ID NO:125	L234F	0.13	0.09	1.20	0.35	0.25	1.02	1.94
121	SEQ ID NO:126	L235D	0.04	0.90	1.51	0.71	1.88	0.77	1.26
122	SEQ ID NO:127	L235S	0.12	0.74	1.61	0.64	0.83	0.99	1.04
123	SEQ ID NO:128	L235N	0.03	0.77	1.56	0.76	0.34	1.10	1.46
124	SEQ ID NO:129	L235Q	0.06	0.82	2.33	0.82	0.89	0.89	1.24
125	SEQ ID NO:130	L235T	0.10	0.63	1.39	0.72	1.40	0.93	0.92
126	SEQ ID NO:131	L235H	0.05	1.27	3.86	1.72	0.14	0.88	1.19
127	SEQ ID NO:132	L235Y	0.09	0.79	2.43	0.61	1.09	0.58	1.50
128	SEQ ID NO:133	L235I	0.20	0.24	1.91	0.22	1.16	1.24	0.68
129	SEQ ID NO:134	L235V	0.22	8.80	3.69	2.59	0.91	2.70	1.04
130	SEQ ID NO:135	L235F	0.09	18.07	1.78	1.31	0.79	0.92	1.26

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VW 7/24/07 **Please delete Table 69 beginning at page ~~147~~ 149 and replace with the following rewritten table:**

--Table 69

Variant	SEQ ID NO	Substitution(s)	FcγRI	FcγRIIa	FcγRIIb	FcγRIIc	FcγRIIIa	FcRn	C1q
107	SEQ ID NO:112	A330Y/I332E	3.14	5.40	2.90	3.84	19.83	2.85	1.15
109	SEQ ID NO:114	A330L/I332E	6.44	1.58	1.16	1.58	21.23	5.36	1.08
167	SEQ ID NO:172	L328E/I332E	0.91	8.59	5.54	10.21	1.85	0.31	
171	SEQ ID NO:176	L328T/I332E	1.42	3.07	10.28	22.69	4.51	0.84	
174	SEQ ID NO:179	L328A	0.80	4.24	1.08	1.11	0.84	1.06	

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VW 7/24/07 **Please delete the paragraph beginning at page ~~147~~ 149, paragraph [240] and replace with the following rewritten paragraph:**

--[240] These data show even more convincingly that it is possible to tune Fc for Fc ligand specificity, often by using very subtle mutational differences. For example, the A330Y/I332E variant (SEQ ID NO:112) enhances binding to all FcγRs, particularly FcγRIIIa, as well as FcRn, while maintaining binding to C1q. However the ~~A300L~~A330L/I332E variant (SEQ ID NO:114) shows enhanced binding to FcγRI and FcγRIIIa, but has WT affinity for the FcγRII's. In contrast, mutations at L328 provide preferential